bio-protocol Preprint

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Bioinformatics analyses

KH Karin Hansson DB Daniel Bexell

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An abbreviated version of this protocol was published in Science Translational Medicine in Sep 2020

Therapeutic targeting of KSP in preclinical models of high-risk neuroblastoma

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Detailed protocol

Analysis of publicly available datasets of patient tumors

1. Publicly available datasets were obtained from and analyzed by R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl). The datasets SEQC498 and Versteeg88, containing 498 and 88 neuroblastomas respectively, were used for analysis of the *KIF11* gene expression.
2. The expression in neuroblastoma (Versteeg88) was compared to various normal tissue (Roth 504) and normal tissue from adrenal gland (Various 13) as well as the largest available datasets from other cancer diagnoses across platforms hs, u133p2, MAS5.0. Within neuroblastoma, expression of *KIF11* was compared between high and low risk tumors (SEQC498) or between INSS stages (Versteeg88).
3. Statistical analyses were performed using two-sided unpaired t-tests. For analysis of overall survival between high and low *KIF11* expression in Kaplan-Meier curves, the median was used as a cut-off and statistical significance was calculated using the log-rank test.

Expression of *KIF11* in cell lines

1. Expression of *KIF11* in cell lines was extracted from the Cancer Cell Line Encyclopedia (CCLE, https://portals.broadinstitute.org/ccle). CERES(*31*) and DEMETER2(*32*) dependency scores were obtained from Broad Institute's DepMap portal (https://depmap.org/portal/) by downloading the CRISPR dataset (DepMap Public 19Q3, Dataset doi:10.6084/m9.figshare.9201770.v1.) and the combined RNAi dataset (DEMETER2 Data v5).
2. Tumor types with data from less than five cell lines were excluded from subsequent analyses. Publicly available data on IC_{50} values of ARRY-520 were obtained from the genomics of drug sensitivity in cancer (GDSC) database (https://www.cancerrxgene.org).

RNA sequencing

1. The mRNA library preparation was performed with TruSeq® Stranded mRNA Library Prep (Illumina, San Diego, CA) on the King Fisher FLEX system (Thermo Fisher Scientific, Waltham, MA).
2. NextSeq 500 (Illumina) was used for sequencing using the NextSeq 500/550 High Output v2.5 kit (Illumina).
3. Reads were aligned to the human GRCh38 (ENSEMBL database) reference genome sequence and the annotation (GTF) from release 94 using HISAT2 software.
4. Gene level assembly and quantification were performed using StringTie, and raw counts were normalized using DESeq2.
5. All data were uploaded to the R2: Genomic Analysis and Visualization Platform (http://r2.amc.nl) under the name PDX Neuroblastoma KSPi-in-vitro_20190416 - Aaltonen - 16 - deseq2 - ensh38e94.
6. All RNA-seq analyses were performed on the R2 platform.
7. Heat maps of the differentially expressed genes between treatment group and controls were identified by one-way analysis of variance (ANOVA, $p<0.01$) with false discovery rate (FDR) correction for multiple testing.
8. Gene ontology analysis was performed with 2x2 contingency table analysis (chi-square) with continuity correction.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Hansson, K. and Bexell, D. (2020). Bioinformatics analyses. Bio-protocol Preprint. bio-protocol.org/prep548.
2. Hansson, K., Radke, K., Aaltonen, K., Saarela, J., Mañas, A., Sjölund, J., Smith, E. M., Pietras, K., Påhlman, S., Wennerberg, K., Gisselsson, D. and Bexell, D.(2020). Therapeutic targeting of KSP in preclinical models of high-risk neuroblastoma . Science Translational Medicine 12(562). DOI: 10.1126/scitranslmed.aba4434